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Subject **Leaching of residual peroxide from a gelcoat cured with [redacted]**

CONFIDENTIAL

[redacted]

Summary

A reversed phase HPLC method for the determination of type 3 and type 4 Methylisopropyl-ketone peroxides was successfully developed. The method features an in-line solid phase extraction step to concentrate the injected peroxides, a gradient reversed phase separation step to separate the peroxides and a post column reaction in which N,N-dimethyl-p-phenylene diammonium-dichloride (DMPD) is oxidized by the peroxide to an intensely colored red compound which is detected by light absorption at 554 nm.
The method is highly selective, yields linear calibration lines, offers a good precision and offers low detection (LOD) and quantitation (LOQ) limits:

	Precision			
	conc., µg/l	sd., %	LOD, µg/l	LOQ, µg/l
Ultrapure water				
T4	60.2	0.60	0.23	0.76
T3-1	11.9	1.58	0.24	0.80
T3-2	11.9	1.91	0.25	0.83
Seawater simulant				
T4	53.1	0.36	0.42	1.41
T3-1	10.5	0.83	0.43	1.45
T3-2	10.5	0.76	0.48	1.59

Using this method, the leaching of Methylisopropylketone peroxides from a gelcoat manufactured with use of [redacted] was investigated. The gelcoat was contacted during 10 days with ultrapure water or with seawater simulant at a temperature of 22°C, applying an area-to-volume ratio of 6 dm^2/l. A higher test temperature (e.g. 40°C) was not feasible, since the peroxides then decomposed too rapidly. No Methylisopropylketone peroxides were detected in the contact liquids.

[redacted]

1. Introduction

[redacted] is a high reactive methyl isopropyl ketone peroxide (MIPK) which is particularly suitable for the curing of gelcoat resins and laminating resins used for the production of boat hulls and deck parts. It is important that the gelcoat does not release any residual peroxide when brought in contact with (sea)water, since this may cause damage to the marine environment. At the request of the Belgian authorities, a leaching study was conducted to investigate this aspect.

2 Experimental section

2.1 Samples, standards and chemicals

Samples

Six glass fiber reinforced polyester sheets, length 27 cm, 11 cm wide, thickness 2.5 mm; each bearing on one side a gelcoat with a thickness of 0.45 mm.
Butanox P-50 (approx. 2 mass%) was used for the curing of both the polyester and the gelcoat.

Standards

Methylisopropylketone peroxide (MIPK), batch BOE07059, manufactured by [redacted] was used as a peroxide standard for the quantitative measurements. The Certificate of Analysis of the batch is illustrated in appendix 1.
Essentially, MIPK is a solution of approximately 20 mass% 1,2-Dimethylpropylidene dihydroperoxide (T4 peroxide) and 7.5 mass% 1,1'-Dioxybis(1,2-dimethylpropyl) dihydro-peroxide (T3 peroxide) in Dimethylphthalate. T3 consists of two stereoisomers, which are easily separated when analyzed by HPLC.

Chemicals

Acetonitrile	:	Ultra gradient HPLC grade, J.T. Baker, Deventer, The Netherlands
Water	:	Ultrapure : resistivity > 10 MΩ·cm, TOC < 10 ppb, purified with a Milli-Q apparatus, Millipore, Billerica (MA), USA
Sodium chloride	:	Analytical grade, J.T. Baker, Deventer, The Netherlands
AmmoniumIron(II)sulfate.6aq	:	J.T. Baker, Deventer, The Netherlands
DMPD	:	N,N-dimethyl-p-phenylene diammonium-dichloride, Merck, Darmstadt, Germany
Nitric acid	:	1N, analytical grade
Sulfuric acid	:	1N, analytical grade
Seawater simulant	:	30 grams Sodium chloride are dissolved in 1 liter ultrapure water
DMPD reagent	:	dissolve 2000 mg DMPD and 1400 mg Ammonium-Iron(II)-sulfate in 1 liter of 7.8 mM sulfuric acid. The reagent is stored in a dark eluent bottle which is wrapped in Aluminum foil.

[redacted]

2.2 Instrumentation

Standard equipment

- Analytical balance : Mettler AE163, readable to 0.01 mg
- Bulb pipettes : 'Blau' grade, Brand, Germany
- Positive displacement pipettes : Microman, Gilson, France
- Volumetric flasks : 'Blau' grade, Brand, Germany
- 20 ml septum vials : borosilicate glass, with Teflon lined septum and aluminum crimp cap. Thermo Fisher Scientific, The Netherlands.

HPLC apparatus

The HPLC apparatus was assembled from Agilent Model 1100 units, as illustrated in appendix 2.

The core of the HPLC setup consists of a solvent cabinet with 4-port membrane degasser, a quaternary pump, a column oven provided with a 6-port rotary valve and a variable wavelength UV-Vis detector. The core system was used for initial pilot studies and was then extended with a solid phase extraction module and with a post column reactor.
To enable large volume injections, the standard plumbing of the autosampler is replaced by a '900 µl injection upgrade kit' (Agilent, part no. G1363A) and a '5 ml seat capillary' (part no. 0101-0301).

The solid phase extraction module consists of a second quaternary pump and a 20 mm long, 2 mm i.d. precolumn, packed with PLRP-S (10µm, 100Å; Polymer Laboratories, Church Stretton, UK) which is connected to the 6-port valve. Samples are introduced onto the SPE column in counterflow mode, in order to facilitate their rapid desorption and thus prevent peak broadening.

The reactor unit is connected to the outlet of the UV detector via a low-dead-volume T-piece, and consists of PEEK capillary tubing (Fisher Emergo, Landsmeer, The Netherlands) with a length of 15 meters and an inner diameter of 0.01 inch which is accommodated in a separate oven (Mistral, Spark, Emmen, The Netherlands), a binary pump and a second UV-Vis detector.

All units are controlled from an Agilent Model 1100 control module, which allows the fully automated processing of a batch of HPLC runs.

The Atlas chromatography data system (version 7.10.9, Thermo Instruments) was used to record and process the data from both UV-Vis detectors.

Extraction cells

Extraction cells for one-sided contact studies were constructed from 20 ml borosilicate septum vials. The bottom sections of the vials were precisely removed by a professional craftsman.
Between the cell and the polyester test strip, an O-ring is placed to ensure a leak-free connection. The O-rings are cut out of a 50 µm thick Teflon membrane (Millipore, Billerica (MA), USA, Art.nr. FHUP04700).
The cell body, O-ring and test strip are firmly pressed together with a frame clamp.

2.3 Procedures

HPLC conditions

Analytical column	:	Zorbax Rx-C18, 250 x 4.6 mm, dp = 5 µm
Column oven	:	25°C
Reactor oven	:	70°C
Mobile phases		
- pump 1	:	A: Water, B: Acetonitrile. Filtered and degassed
- pump 2	:	Water. Filtered and degassed
- pump 3	:	DMPD reagent
Flow rates		
- pump 1	:	0.7 ml/min
- pump 2 (standby)	:	0.1 ml/min
- pump 3	:	0.4 ml/min
Injection volume	:	1 ml
Detection	:	UV at 220 nm; Vis at 554 nm

Time table

0 min	:	pump 1 : 50 % B
0.01 min	:	pump 2 : 1.2 ml/min
5 min	:	switch valve from : 'load SPE' to : 'inject'
5 min	:	pump 1 : 50 % B, linear ramp
15 min	:	100 % B
18 min	:	100 % B
19 min	:	50 % B
23 min	:	50 % B

Calibration lines

Stock solution A:
accurately weigh 30 mg of the MIPK standard, add 250 µl Acetonitrile, transfer the mixture to a 100 ml volumetric flask and add ultrapure water or seawater simulant to the mark. Mix.
Note: the addition of Acetonitrile seemed essential - without Acetonitrile a slightly hazy solution was obtained, indicating that the MIPK sample has not completely dissolved.

Solution B:
dilute 1 ml of solution A to 1 l with ultrapure water or with seawater simulant.
Using accurate pipettes and volumetric flasks, prepare further dilutions.

Peroxide stability test

To inactivate any biological contaminants, pipettes, volumetric flasks, 20 ml vials and septa were immersed in boiling ultrapure water for 30-60 minutes and were dried in an oven at 120°C. Ultrapure water and seawater simulant were boiled before use.
Standard solutions containing 1.2 mg/l of the Methylisopropylketone peroxide standard were prepared in ultrapure water and in seawater simulant.
Two groups of 20 ml vials were loaded with 5 ml of a standard solution. Using crimp caps, the vials were sealed with a Teflon-lined septum and were stored protected from daylight at three well controlled temperatures: 4 ± 2°C; 22 ± 2°C and 40 ± 2°C, for a period of up to two weeks. At predetermined times, the content of one of the vials was transferred to a 5 ml

autosampler vial and the peroxide content was determined, relative to a freshly prepared MIPK standard solution.

Leaching test

Rectangular test strips with dimensions of approximately 4 x 4 cm were cut out of the polyester sheets, thereby avoiding to take a sample at the very edges of the sheet.

To inactivate any biological contaminants, pipettes, volumetric flasks, extraction cells and septa were immersed in boiling ultrapure water for 30-60 minutes and were dried in an oven at 120°C. Ultrapure water and seawater simulant were boiled before use.

Using crimp caps, the extraction cells were sealed at their upper side with a Teflon-lined septum. While holding the cell in an inverted position, 5.24 ml of test liquid was introduced in the cell, the O-ring was mounted, the test strip was put in place and the ensemble was inserted in a frame clamp. For control experiments, e.g. blanks, the Teflon O-ring was replaced by an intact Teflon membrane.

The cells were inverted to allow the test liquid to contact the polyester test strip (or the membrane) and were stored, protected from daylight, at 22 ± 1°C for a period of up to 10 days.

At predetermined times, one of the cells was opened, the test liquid was transferred to a 5 ml autosampler vial and its peroxide content was determined, relative to a freshly prepared MIPK standard solution.

Leaching tests were performed with different test liquids:

- ultrapure water and seawater simulant were contacted with the test strips to determine the amounts of T3 and T4 peroxide released by the gelcoat.
- MIPK standard solutions containing 50 µg/l T4 peroxide, 10 µg/l T4 peroxide and/or 10 µg/l of each T3 isomer were contacted with the test strips to prove that these concentrations would have been detected if such amounts of peroxide had leached from the gelcoat.

3 Results and discussion

The T3 and T4 methylisopropylketoneperoxides (MIPK) forming the active ingredients from [redacted] are expected to react completely during the curing process, especially since the added catalyst is not consumed. A leaching study was performed to validate this supposition.

At the start of the study it was planned to conduct the measurements by contacting a gelcoat during 10 days at a temperature of 40°C with a seawater simulant and also with ultrapure water, applying an area-to-volume ratio of 6 dm^2/l.
An Ecotox specialist was consulted concerning the detection limit (LOD) of the method to be developed for peroxide analysis. A LOD of ≤ 0.3 mg/l (0.3 ppm) was advised for leaching determinations conducted at 40°C. Lower test temperatures would require lower detection limits. E.g. for determinations conducted at room temperature, a LOD of ≤ 10 µg/l (10 ppb) was recommended.

3.1 Pilot tests

Before commencing with the leaching study several pilot tests were conducted first, using a non-optimized reversed phase HPLC method with UV detection and comparatively high (100 - 250 mg/l) peroxide concentrations.
These tests were aimed at : obtaining suitable criteria for HPLC method development, checking the stability at several temperatures of aqueous solutions of an MIPK standard and checking the possible presence in extracts from the gelcoat of matrix impurities which might interfere with the peroxide peaks.
The outcome of the pilot tests revealed several problems. At room temperature the peroxide concentrations remained fairly stable during storage for 10 days, but at 40°C a severe loss of the peroxides occurred. Attempts to reduce this degradation, for instance by switching to storage vials from alternative suppliers or by modifying the vial cleaning protocol offered only a limited improvement. It was concluded that the leaching study had to be performed at room temperature.
In addition, serious coelution problems were observed. When applying UV detection at 220 nm, baseline irregularities induced by impurities present in the mobile phase as well as peaks of matrix impurities extracted from the gelcoat were detected over a broad retention time span - including the retention positions of the peroxides. These effects appeared to obscure the peroxide peaks already at the 1-10 ppm concentration level. It was not possible to establish HPLC conditions which provided an adequate resolution for the peroxide peaks. The use of a highly selective detection technique instead of UV detection seemed therefore mandatory. It appeared that HPLC-MS was not an option. The methylisopropylketone peroxides could not be ionized with adequate sensitivity.

3.2 Method development and validation

Resolution and specificity

A dedicated HPLC method was developed which combined an in-line solid phase extraction step to concentrate the injected peroxides, a gradient reversed phase separation step to separate the peroxides and a post column reaction in which N,N-dimethyl-p-phenylene-diammonium-dichloride (DMPD) is oxidized by the peroxide to an intensely colored red compound which is detected by light absorption at 554 nm.
For solid phase extraction, PLRP-S was selected as the sorbent. On this sorbent, the T3 and T4 methylisopropylketoneperoxides are quantitatively trapped. This was checked by

comparing the peak areas obtained from a 1 ml water sample containing 1 mg/l of the MIPK standard which was injected using the SPE unit and from a 10 µl sample containing 100 mg/l of the MIPK standard which was injected directly onto the HPLC column. Both approaches yielded similar peak areas - as would theoretically be expected.

Optimal, robust conditions for the post column reaction had already been assessed in a separate study. The Fe(II) catalyzed reaction of peroxides with N,N-dimethyl-p-phenylene-diammonium-dichloride (DMPD) is best conducted at 70°C, in combination with a reaction time of approximately 42 seconds.
To check the robustness of the reaction when applied to the T3 and T4 peroxides, the residence time in the capillary reactor was varied by adjusting the flow rate through the reactor. Changes of up to 50% did not significantly affect the reaction yield : the measured peak areas were found to vary inversely proportional with the flow rate, as is predicted by theory for a UV detector.
The addition of the capillary reactor to the HPLC system has an unavoidable side-effect: it causes some peak broadening. The broadening can easily be determined by comparing the signals from respectively the UV 220 nm detector, which is positioned directly after the HPLC column, and the VIS 554 nm detector, which is positioned after the reactor. It was found that the width of the peroxide peaks is increased by approximately 45%.
The performance of the reactor was monitored daily by injecting freshly prepared standard solutions containing 0.25 mg/l MIPK dissolved in water. It was noticed that reactor fouling occurred after several days of use. The peroxide peaks then started to develop tailing and the reaction yield appeared to decrease. The performance of the reactor was restored by rinsing the capillary with 1 N Nitric acid for at least 8 hours.

Chromatograms recorded with the final version of the HPLC method are illustrated in appendix 3.
The chromatogram recorded at 220 nm illustrates the retention positions of the main compounds and the impurities which are present in a 303 mg/l solution of MIPK in water. The most dominant peak originates from Dimethylphthalate, owing to its high concentration and its comparatively high molar extinction coefficient. The peak shows a distinct tailing, which causes the baseline after the peak to drift significantly for several minutes. The T4 peroxide peak displays a slight overlap with a neighboring impurity; the two T3 isomer peaks are well resolved.
The chromatogram recorded at 554 nm from a 1.7 mg/l solution of MIPK in water displays exclusively the three peroxide peaks, thus demonstrating the high selectivity and sensitivity of the post-column reaction. All peaks are adequately resolved.

Linearity and precision

To check the linearity of the developed method, calibration lines were measured which cover the concentration range of 1-80 µg/l (ppb) for T4 peroxide and 1-15 µg/l (ppb) for each T3 isomer. The calibration lines are illustrated in appendix 4. All lines appear to be linear and have acceptable correlation coefficients.
To determine the precision of the method, two of the calibration solutions were injected seven times. The measured data are tabulated in appendix 5.
The tested solutions contain approximately 55 ppb T4 peroxide and approximately 11 ppb of each T3 peroxide isomer.
For the calibration solution prepared in water, relative standard deviations were measured of respectively 0.6% for T4, 1.8% for T3-1 and 1.8% for T3-2.
For the calibration solution prepared in seawater simulant, relative standard deviations were measured of respectively 0.3% for T4, 1.2% for T3-1 and 1.2% for T3-2.

The determined precision of the developed HPLC method is judged to be good.
The difference between the values obtained with respectively water and seawater simulant may reflect the fact that the samples were analyzed on two different days.

Detection limit (LOD), Quantitation limit (LOQ)

Typical chromatograms from standard solutions containing approximately 20 µg/l T4 and 5 µg/l of each T3 isomer are illustrated in appendix 6. From these chromatograms, the detection and quantitation limits of the method were determined according to a procedure taken from the European Pharmacopoeia (ref.5.1).

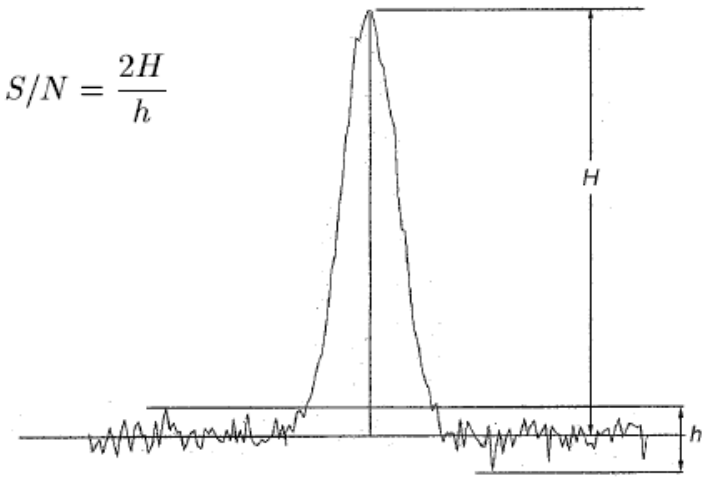


Figure 1. Determination of the S/N ratio.

The height of the peroxide peak and the height of the baseline noise are measured and the signal-to-noise ratio (S/N) is determined as illustrated in figure 1.

The LOD and LOQ values are defined as:
LOD : concentration corresponding to a S/N ratio of 3.
LOQ : concentration corresponding to a S/N ratio of 10.

For the sample prepared in water, LOD values of 0.23 ppb, 0.24 ppb and 0.25 ppb were calculated for respectively T4, T3-1 and T3-2 peroxide. The corresponding LOQ values are respectively 0.76, 0.80 and 0.83 ppb.
For the sample prepared in seawater simulant, LOD values of 0.42 ppb, 0.43 ppb and 0.48 ppb were calculated for respectively T4, T3-1 and T3-2 peroxide. The corresponding LOQ values are respectively 1.41, 1.45 and 1.59 ppb.
The higher values obtained with seawater simulant are caused by a small ripple affecting the baseline, which was absent during analysis of the water samples.

3.3 Peroxide stability tests

The developed HPLC method was first applied to check once more the stability of the T3 and T4 peroxides during storage of their solutions at several temperatures. The selected peroxide concentrations were now substantially lower than those used for the pilot tests.
Solutions containing 1.2 mg/l MIPK were prepared in ultrapure water and in seawater

simulant and were stored during two weeks at 4°C, 22°C or 40°C. Their peroxide contents were analyzed with intervals of several days. The results are listed in appendix 7.
The conclusions drawn from the pilot tests were confirmed. At 4°C, the peroxides remained stable for two weeks. At 40°C, an unacceptably large degradation of the peroxides occurred. Most of the T4 peroxide was destroyed after two weeks storage, the T3 peroxides were even almost completely degraded within only seven days. At 22°C, T4 peroxide remained stable whereas T3 peroxide was nearly halved.
The observation that the most severe peroxide loss occurs at the highest test temperature indicates that the loss is caused by chemical degradation of the peroxides and not by their adsorption, since adsorption is normally seen to have the largest impact at the lowest test temperature.
In view of the rapid degradation of the peroxides at 40°C it was decided to perform the leaching tests on the gelcoat at 22°C.

3.4 Leaching tests

A gelcoat, deposited on polyester test strips, was contacted at 22°C during 10 days with either ultrapure water or seawater simulant, applying an area-to-volume ratio of 6 dm^2/l.
The peroxide content of the contact liquids was analyzed with intervals of several days.
The results are illustrated in appendices 8 and 9.
In none of the samples any peroxide was detected, implying that the peroxide concentration in the contacted liquids remained below the detection limit of 0.5 µg/l (0.5 ppb).

To validate the proper functioning of the HPLC system, a control experiment was conducted in which a group of test strips were contacted during 10 days with an MIPK standard solution instead of with ultrapure water or with seawater simulant. MIPK solutions containing 50 µg/l T4 peroxide, 10 µg/l T4 peroxide or 10 µg/l of each T3 isomer were used.
Aliquots from these MIPK solutions were analyzed in parallel with the samples from the ultrapure water and seawater simulant contact experiments.
The results are illustrated in appendix 9. When compared to the outcome of the stability test (appendix 7), contact with the gelcoat appears to promote peroxide degradation significantly. After 10 days of contact, the T4 peroxide content from the 50 µg/l solution is almost halved (stability test: T4 stable). Only 20% of T3 peroxides and 20 - 50% of the T4 peroxide from the 10 µg/l solution are recovered after 6 days of contact (stability test: T3 halved after 10 days). The chromatograms illustrated in appendix 9 however show that the 10 ppb additions of T4 peroxide and T3 peroxide still produce well detectable peaks after 6 days of contact. This demonstrates convincingly that these low concentrations would certainly have been detected in the contact liquids from the leaching tests with ultrapure water and seawater simulant (appendix 8), in case any MIPK peroxides had leached from the gelcoat in large enough quantities to build up a concentration of approximately 2 ppb T3 or T4 peroxide in that contact liquid.

[redacted]

4. Conclusions

- A reversed phase HPLC method for the determination of type 3 and type 4 methylisopropylketone peroxides was successfully developed. The method is highly selective, yields linear calibration lines, offers a good precision and offers low detection (LOD) and quantitation (LOQ) limits:

	Precision			
	conc., µg/l	sd., %	LOD, µg/l	LOQ, µg/l
Ultrapure water				
T4	60.2	0.60	0.23	0.76
T3-1	11.9	1.58	0.24	0.80
T3-2	11.9	1.91	0.25	0.83
Seawater simulant				
T4	53.1	0.36	0.42	1.41
T3-1	10.5	0.83	0.43	1.45
T3-2	10.5	0.76	0.48	1.59

- Methylisopropylketone peroxides appear to decompose rapidly at 40°C, but not at 22°C or 4°C.
- A leaching study was conducted at 22°C. A gelcoat manufactured with use of MIPK peroxide was contacted during 10 days with ultrapure water or with seawater simulant. No methylisopropylketone peroxides were detected in these contact liquids.

5. References

5.1 European Pharmacopoeia 5.0, section 2.2.46.

6. Archiving information

[redacted]

7. Appendices

Appendix 1. Certificate of Analysis of the [redacted] standard.

Appendix 2. Scheme of the HPLC apparatus.

Appendix 3. HPLC Chromatograms from solutions of the Methylisopropylketoneperoxide standard in seawater simulant, recorded with UV-220nm and Vis-554nm detection.

Appendix 4. Calibration lines and chromatograms recorded at 554 nm from solutions of the Methylisopropylketone peroxide standard prepared with ultrapure water or with seawater simulant.

Appendix 5. Determination of the standard deviation of the HPLC method from multiple injections of two Methylisopropylketone peroxide standard solutions prepared with respectively ultrapure water and seawater simulant.

Appendix 6. Chromatograms recorded at 554 nm from Methylisopropylketone peroxide standard solutions containing 22 µg/l T4 peroxide and 5 µg/l of each T3 peroxide isomer.
Detection limits and quantitation limits of the HPLC method.

Appendix 7. Stability of 1.2 mg/l solutions of a Methylisopropylketone peroxide standard when stored at resp. 40°C, 22°C and 4°C for a period of up to 13 days.

Appendix 8. Chromatograms recorded at 554 nm from samples of ultrapure water or seawater simulant which have been contacted at 22°C with the gelcoat during 1, 3 or 10 days.

Appendix 9. Recoveries of methylisopropylketone peroxides from MIPK standard solutions which have been contacted with the gelcoat at 22°C for up 10 days, and typical chromatograms, recorded at 554 nm, obtained after a contact period of 6 days.

[redacted]

Appendix 1. Certificate of Analysis of the Methylisopropylketoneperoxide standard.

[redacted]

Product name : -

Chemical name : [redacted]

Batch number : [redacted]

Test results:

Method	Analysis of	Unit	Result *1
Jo/72.10, Jo/72.11, Jo/02.1	Peroxidic compounds (sum) *See page 2 for a specification*	% m/m	28.8 (± 1.0)
HPLC	[redacted]	% m/m	68.0 (± 1.0)
HPLC		% m/m	1.4 (± 0.2)
Amp/88.9	Water	% m/m	1.8 (± 0.2)

*1 bracketed values are estimated 95% confidence intervals

Archive code : [redacted]

Analytical documentation : [redacted]

[redacted]

[redacted]

[redacted]

Appendix 1 - continued

[redacted]

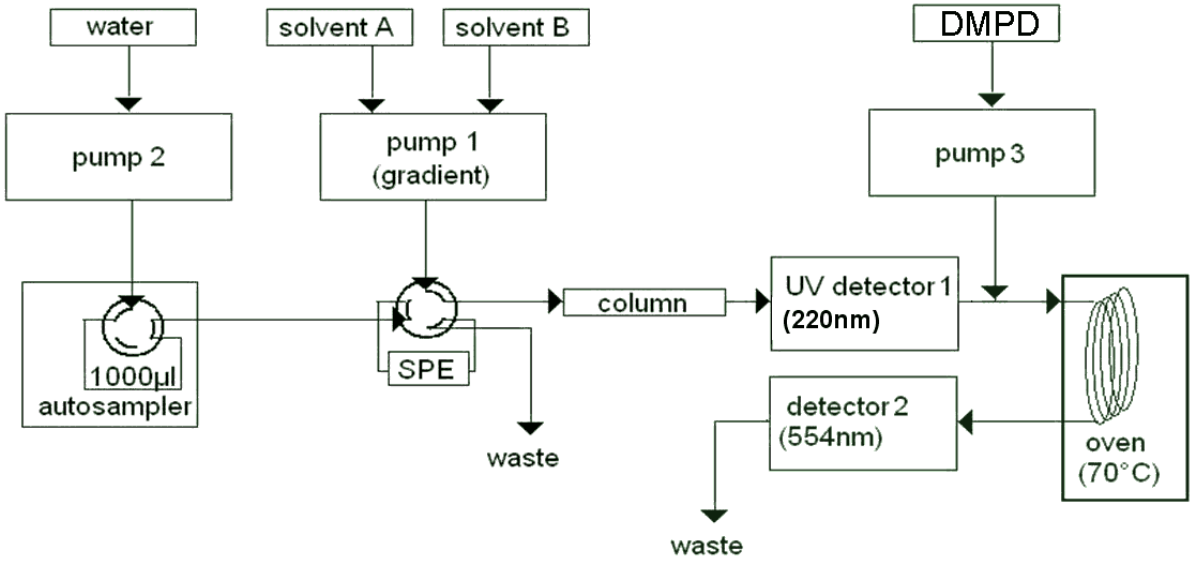
[redacted]

[redacted]

structure	% m/m
[redacted]	

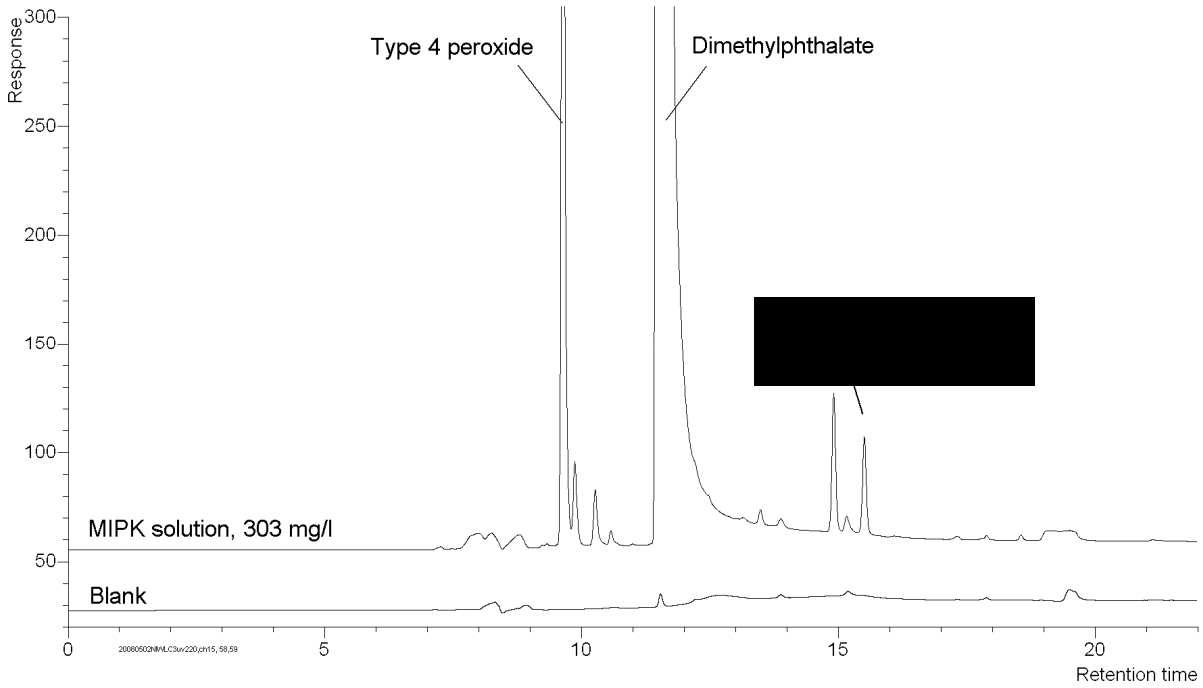
[redacted]

Appendix 2. Scheme of the HPLC apparatus.

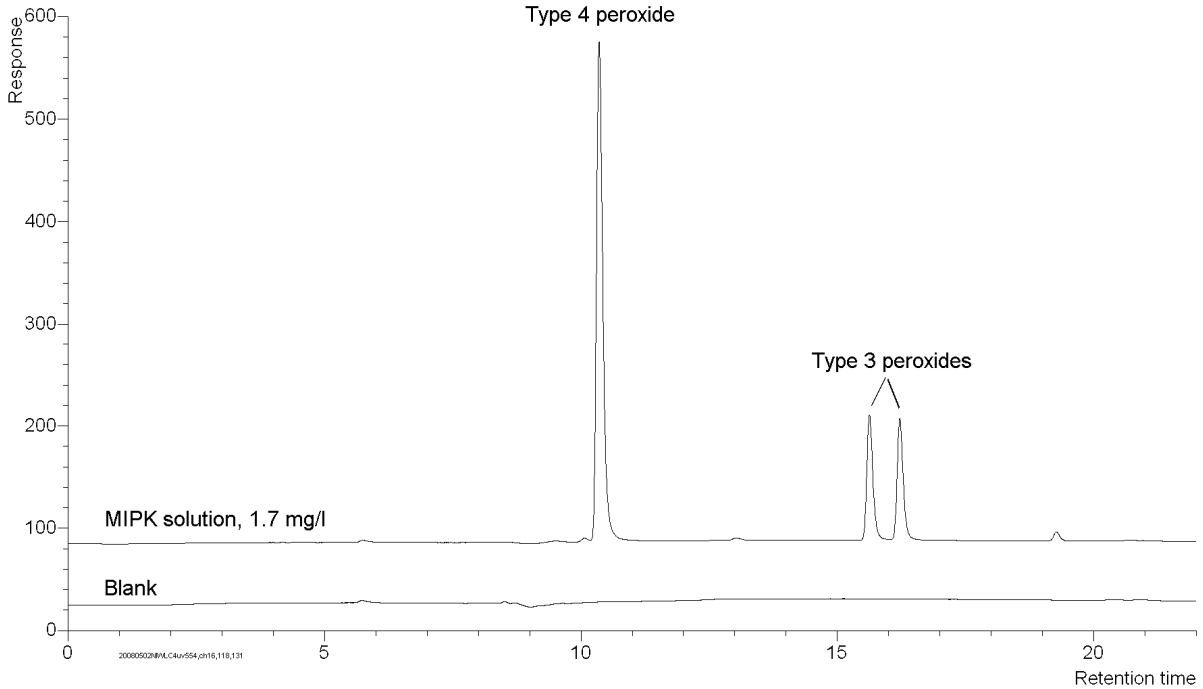


Appendix 3. HPLC Chromatograms from solutions of the Methylisopropylketoneperoxide standard in seawater simulant, recorded with UV-Vis detection.

3A. 303 mg/l, 220 nm, detector positioned after the HPLC column.



3B. 1.7 mg/l, 554 nm, detector positioned after the reactor.



Appendix 4. Calibration lines and chromatograms recorded at 554 nm from solutions of the Methylisopropylketone peroxide standard. The solutions were prepared with ultrapure water or with seawater simulant.

4A. Results from solutions prepared with ultrapure water

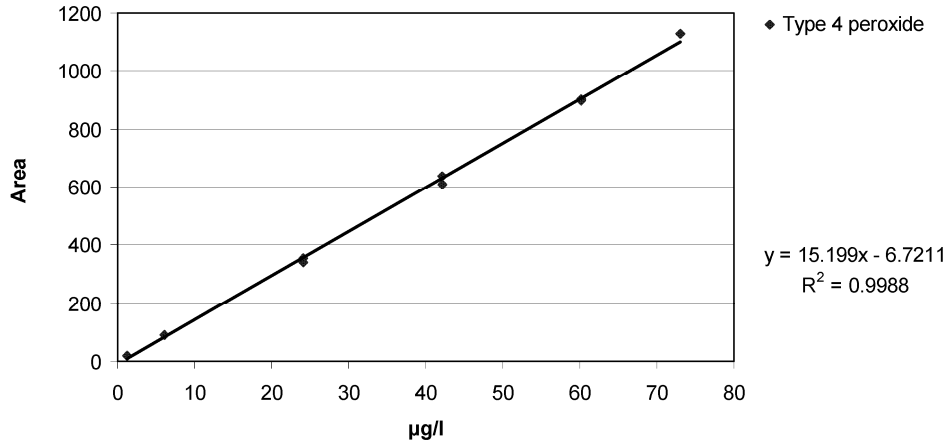
T4		T3-1		T3-2	
µg/l	Area	µg/l	Area	µg/l	Area
1.20	19.0	0.24	= LOD *	0.24	= LOD
1.20	19.3	0.24	= LOD	0.24	= LOD
6.02	87.6	1.19	19.4	1.19	16.6
6.02	92.0	1.19	16.3	1.19	15.3
24.07	339.3	4.78	68.9	4.78	68.3
24.07	356.4	4.78	68.6	4.78	62.7
42.13	611.1	8.36	126.7	8.36	123.9
42.13	640.1	8.36	131.4	8.36	123.9
60.18	902.9	11.94	194.7	11.94	192.8
60.18	899.7	11.94	197.1	11.94	191.0
72.97	1129.0	14.48	233.7	14.48	223.4

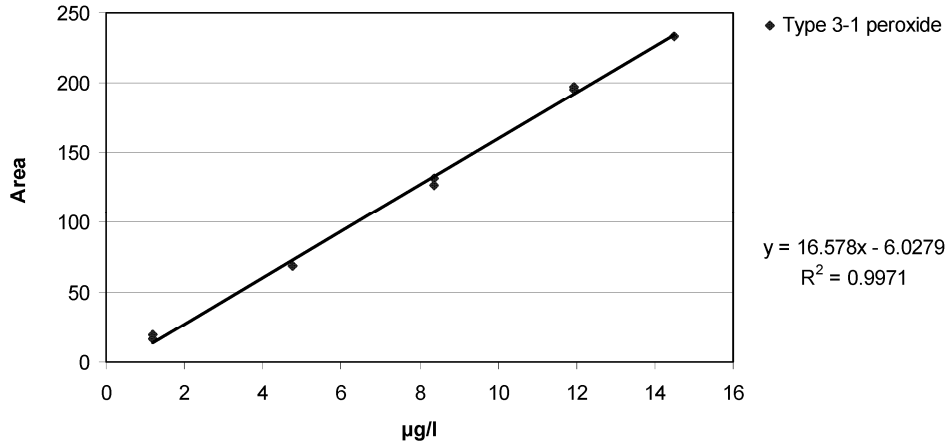
4B. Results from solutions prepared with seawater simulant

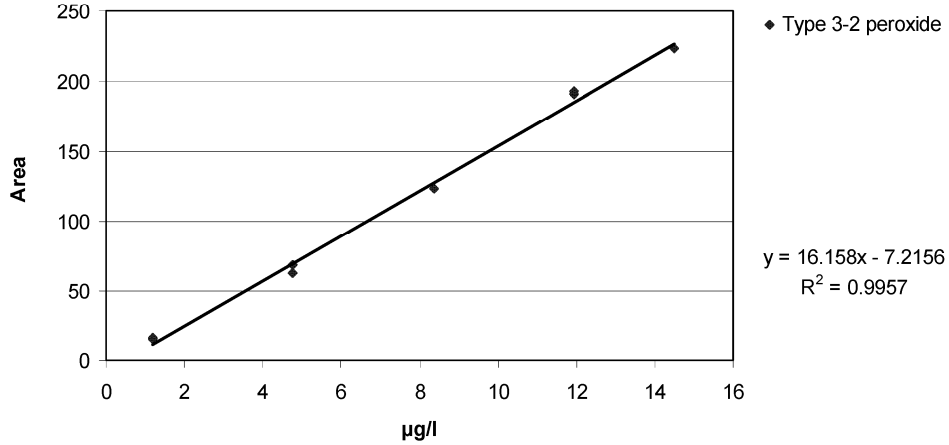
T4		T3-1		T3-2	
µg/l	Area	µg/l	Area	µg/l	Area
1.06	15.5	0.21	< LOD	0.21	< LOD
1.06	11.7	0.21	< LOD	0.21	< LOD
5.31	75.4	1.05	11.9	1.05	11.5
5.31	67.8	1.05	11.9	1.05	9.6
21.26	290.1	4.22	51.9	4.22	48.7
21.26	281.2	4.22	50.3	4.22	46.1
37.20	470.7	7.38	81.4	7.38	77.0
53.14	723.7	10.54	140.0	10.54	129.8
53.14	721.3	10.54	137.6	10.54	131.2
66.09	868.3	13.11	170.3	13.11	158.7

* LOD = detection limit

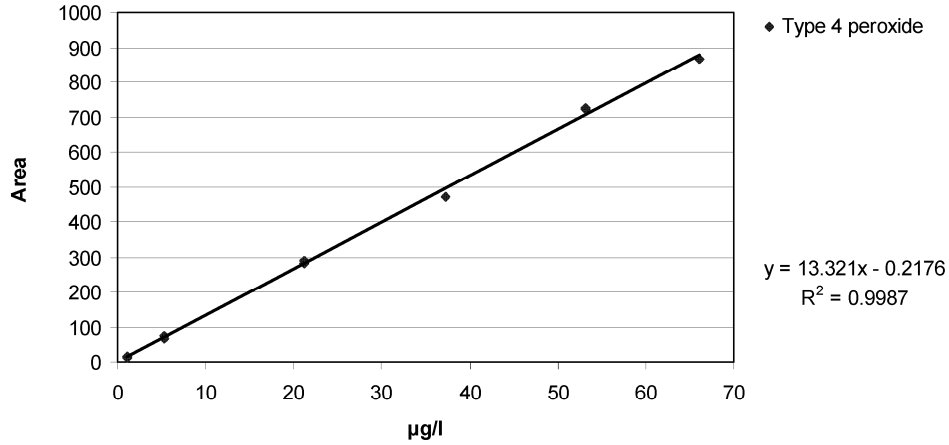
4C. Calibration lines from the standard solutions prepared with ultrapure water

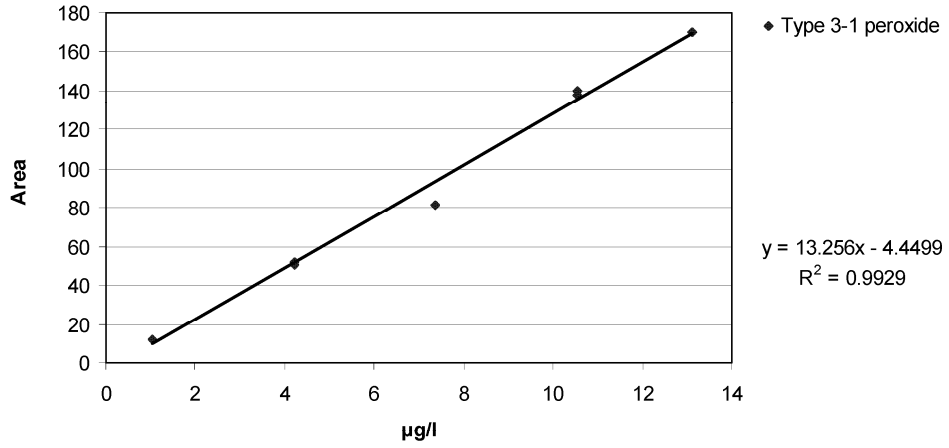


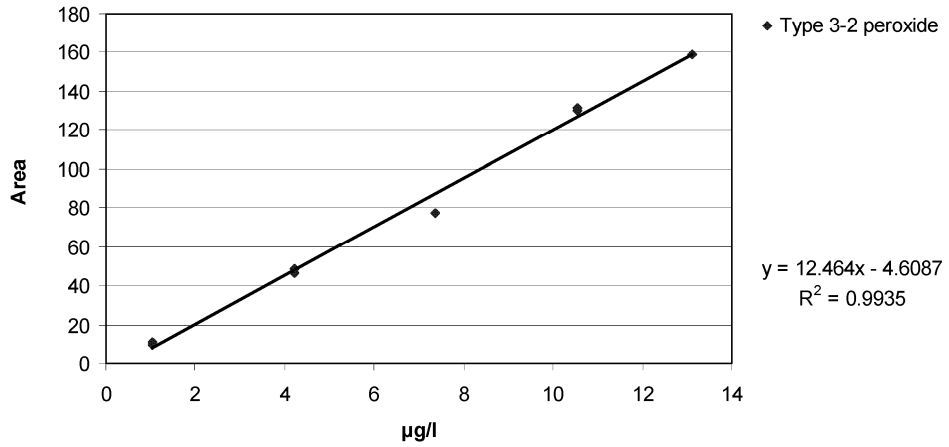




4D. Calibration lines from the standard solutions prepared with seawater simulant







Appendix 5. Determination of the standard deviation of the HPLC method from multiple injections of two Methylisopropylketone peroxide standard solutions prepared with respectively ultrapure water and seawater simulant.

5A. Solution in ultrapure water, containing 60.2 ppb T4 peroxide and 11.9 ppb of each T3 isomer. Detection: 554 nm.

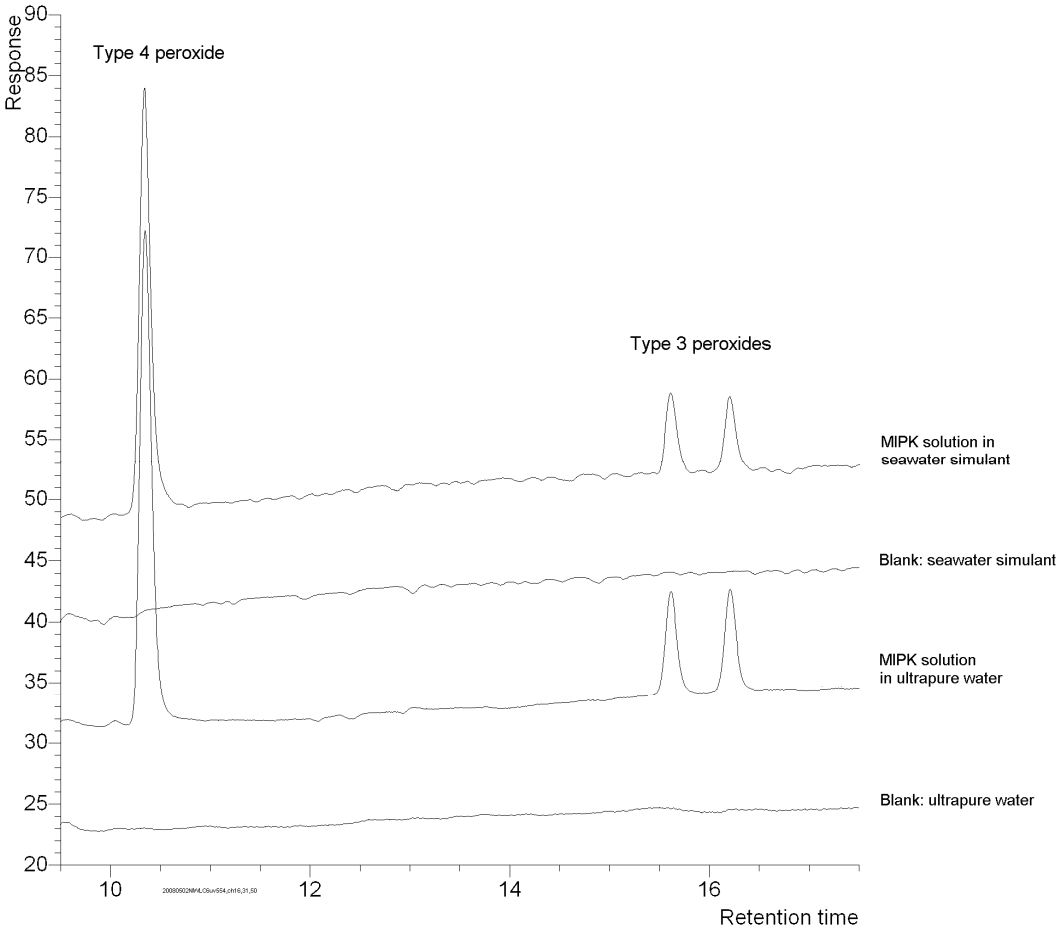
	Area, µV.sec		
Vial nr.	T4	T3-1	T3-2
1	902.9	194.7	192.8
2	899.7	197.1	191.0
3	901.4	201.6	199.6
4	897.6	196.2	187.6
5	907.7	201.7	194.9
6	904.9	194.2	193.8
7	891.1	195.7	192.0
Average	900.8	197.3	193.1
sd.	5.39	3.11	3.70
sd., %	0.60	1.58	1.91

5B. Solution in seawater simulant, containing 53.1 ppb T4 peroxide and 10.5 ppb of each T3 isomer. Detection: 554 nm.

	Area, µV.sec		
Vial nr.	T4	T3-1	T3-2
1	725.1	138.9	131.1
2	723.7	140.0	129.8
3	721.3	137.7	131.2
4	725.8	138.1	131.5
5	719.9	137.6	131.4
6	726.0	138.3	131.2
7	720.4	136.3	133.2
Average	723.2	138.1	131.3
sd.	2.61	1.15	1.00
sd., %	0.36	0.83	0.76

Appendix 6. Chromatograms recorded at 554 nm from Methylisopropylketone peroxide standard solutions containing 22 µg/l T4 peroxide and 5 µg/l of each T3 peroxide isomer.
From these chromatograms, the detection limits and quantitation limits of the HPLC method were determined.

6A. Typical chromatograms of MIPK solutions and solvent blanks.



6B. Calculated detection limits (LOD) and quantitation limits (LOQ).

Ultrapure water	T4	T3-1	T3-2
LOD, µg/l	0.23	0.24	0.25
LOQ, µg/l	0.76	0.80	0.83
Seawater simulant	T4	T3-1	T3-2
LOD, µg/l	0.42	0.43	0.48
LOQ, µg/l	1.41	1.45	1.59

Appendix 7. Stability of 1.2 mg/l solutions of a Methylisopropylketone peroxide standard when stored at resp. 40°C, 22°C and 4°C for a period of up to 13 days. For reference, fresh 1.2 mg/l standard solutions were prepared daily; their T3 and T4 peroxide peak areas were assigned values of 100%.

7A. Solutions in ultrapure water.

T = 4 °C			
Storage time (days)	T4	T3-1	T3-2
2	104	94	98
6	108	101	101
11	100	90	95
11	101	92	86
13	100	97	97
13	98	104	103
T = 22 °C			
Storage time (days)	T4	T3-1	T3-2
1	83	73	72
1	103	86	85
4	94	82	79
4	101	93	89
8	104	85	73
8	114	105	92
11	98	65	50
11	96	65	52
T = 40 °C			
Storage time (days)	T4	T3-1	T3-2
1	86	86	78
1	97	95	92
3	92	32	20
3	94	33	23
7	80	5	3
7	80	8	3

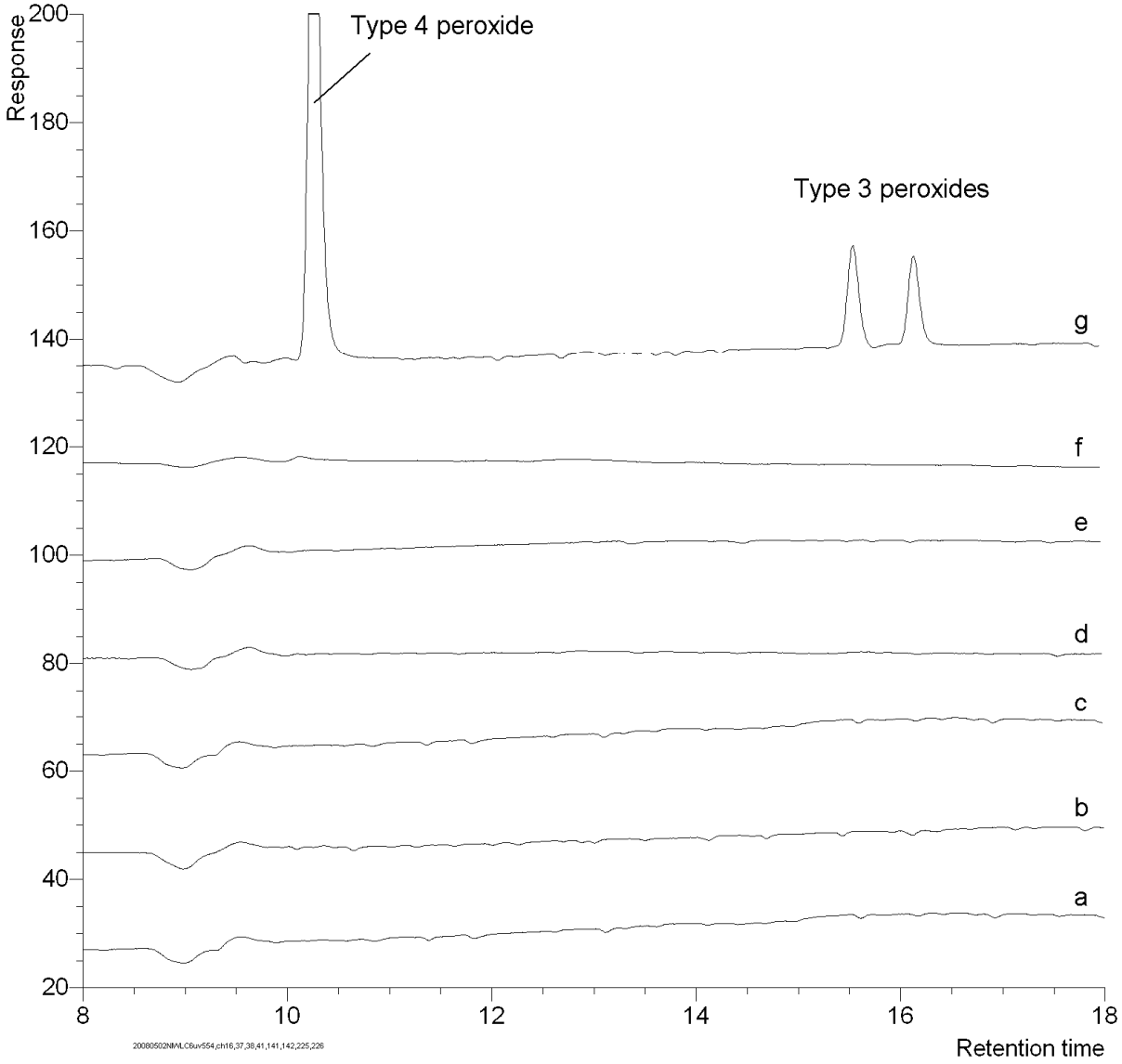
[redacted]

7B. Solutions in seawater simulant.

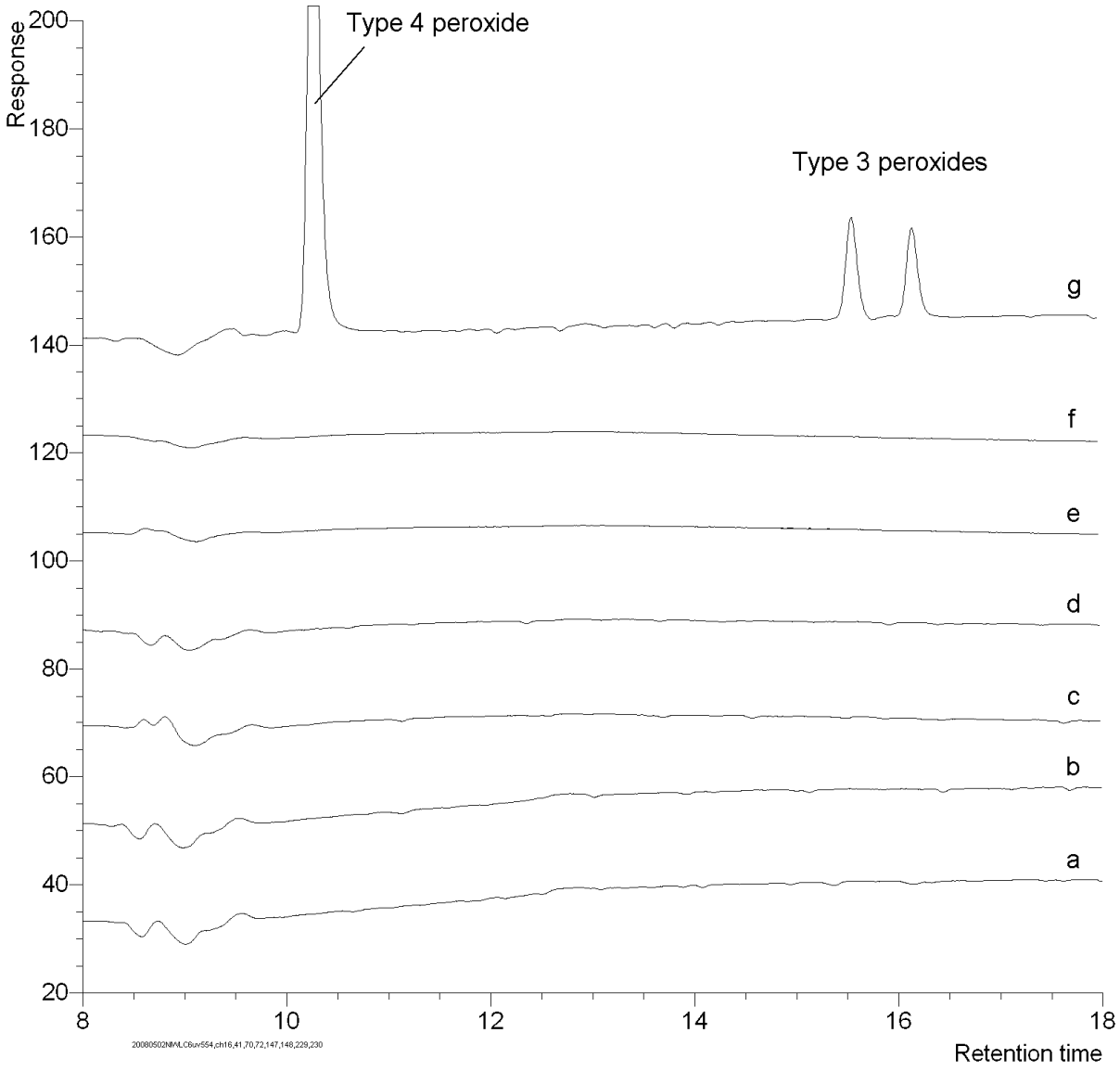
T = 4 °C			
Storage time (days)	T4	T3-1	T3-2
2	102	103	103
6	103	107	107
11	105	117	105
11	99	104	89
13	95	100	100
13	95	105	98
T = 22 °C			
Storage time (days)	T4	T3-1	T3-2
2	105	90	86
6	91	66	51
11	102	70	51
11	108	75	58
13	88	53	43
13	86	42	42
T = 40 °C			
Storage time (days)	T4	T3-1	T3-2
2	78	37	24
6	52	5	1
11	32	1	0
13	28	3	1
13	23	3	1

Appendix 8. Chromatograms recorded at 554 nm from samples of ultrapure water or seawater simulant which have been contacted at 22°C with the gelcoat during 1, 3 or 10 days.
A chromatogram of an MIPK standard solution containing 50 µg/l T4 peroxide and 10 µg/l of each T3 peroxide isomer is also shown (g).

8A. Ultrapure water samples. Contact times: a,b - 1 day; c,d - 3 days; e,f - 10 days.



8B. Seawater simulant samples. Contact times: a,b - 1 day; c,d - 3 days; e,f - 10 days.

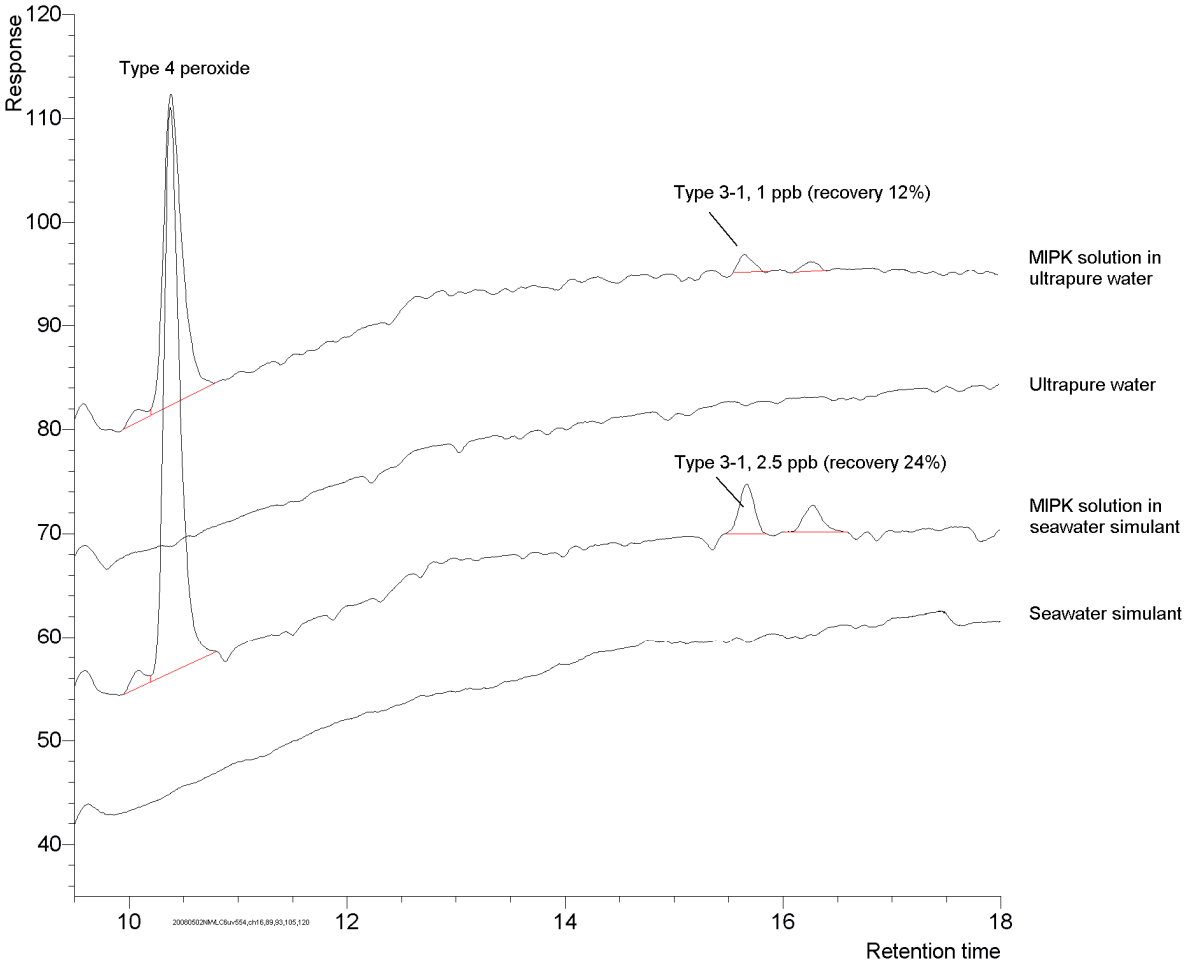


Appendix 9. Recoveries of Methylisopropylketone peroxides from MIPK standard solutions which have been contacted with the gelcoat at 22°C for up 10 days, and typical chromatograms, recorded at 554 nm, obtained after a contact period of 6 days.

9A. Recoveries measured from standard solutions containing 50 µg/l T4 peroxide and 10 µg/l of each T3 peroxide isomer after 1, 3, 6 and 10 days of contact with the gelcoat. For reference, fresh standard solutions were prepared daily; their T3 and T4 peroxide peak areas were assigned values of 100%.

Ultrapure water			
Contact time (days)	T4	T3-1	T3-2
1	96	74	65
1	96	81	73
3	95	83	20
3	95	40	22
6	78	20	20
6	65	19	8
10	80	0	0
10	78	0	0
Seawater simulant			
Contact time (days)	T4	T3-1	T3-2
1	85	68	59
1	73	54	37
3	77	29	13
3	72	23	11
6	57	11	6
6	70	16	10
10	50	0	0
10	56	0	0

9B. Typical chromatograms recorded at 554 nm from samples of ultrapure water, seawater simulant and MIPK standard solutions (initial concentrations: 50 µg/l T4; 10 µg/l T3) after 6 days of contact with the gelcoat.



9C. Typical chromatograms recorded at 554 nm from samples of ultrapure water, seawater simulant and MIPK standard solutions (initial concentration: 10 µg/l T4) after 6 days of contact with the gelcoat.